Abstract

Differential dynamic microscopy combines dynamic light scattering and microscopy techniques for investigation of soft matter dynamics. It is based on observing intensity fluctuations, scattered by an illuminated sample. Series of microscope images is taken with a high speed camera. Images are Fourier analyzed in order to obtain relevant information about particle dynamics in the sample. Different from conventional dynamic light scattering, this method does not require any special experimental requirements, it uses ordinary white-light microscope and an incoherent light source.
1 Introduction

Microscopy and light scattering are important techniques in many areas of science, but they are fundamentally different. Microscopy works in real space, which gives us direct information about a small part of the studied sample. On the other hand, scattering works in reciprocal space, where angular and time dependence of scattered light are observed. For example, dynamic light scattering (DLS) is a method for measuring auto correlation function of scattered intensity in order to recover information about sample dynamics. By combining the two techniques, a very useful tool for collecting information about system dynamics and structure is obtained. However, light scattering usually requires a coherent light source, for example a laser, so scattering experiments were not performed on ordinary white-light microscope. Not until 2008, when Roberto Cerbino and Veronique Trappe proposed a method called differential dynamic microscopy (DDM) \cite{cerbino2008differential} in which they perform exactly that. According to Cerbino and Trappe it is possible to perform a DLS experiment on a weakly scattering object with just an ordinary camera equipped white-light microscope without any special experimental requirements. In this paper I present DDM, show how it can be used to recover information about the observed sample, explain major differences with DLS, and discuss possible uses.

2 Intermediate scattering function

Let us consider a set of \( N \) non-interacting particles, each with coordinate \( r_j(t) \), where \( t \) is time and \( j = 1, ..., N \) is the particle index. Probability density for a particle to undergo displacement \( \mathbf{R} \) in time \( t \) is described by the self-part of the van Hove self-space time correlation function, named after Leon Van-Hove \cite{van1954time, hove1954time}:

\[
G_S = \frac{1}{N} \sum_{j=1}^{N} \delta(\mathbf{R} - [r_j(t) - r_j(0)]),
\]

where \( \langle \cdot \rangle \) represents ensemble average and \( \delta \) is the three-dimensional Dirac delta function. It is called the self part because other \( N - 1 \) particles that could be found at point \( \mathbf{R} \) are not counted in this case. It is related to a quantity that is directly accessible with light and neutron scattering experiments: the self-intermediate scattering function (self-ISF) \( F_S \), by a simple Fourier transformation

\[
F_S(\mathbf{q}, t) = \frac{1}{N} \int d^3re^{i\mathbf{q}\cdot\mathbf{r}} \sum_{j=1}^{N} \delta(\mathbf{R} - [r_j(t) - r_j(0)]) = \frac{1}{N} \left( \sum_{j=1}^{N} e^{-i\mathbf{q}\Delta\mathbf{r}} \right),
\]

where \( \Delta\mathbf{r} = r_j(t) - r_j(0) \). If we write the number density of particles with the Dirac delta function as:

\[
\rho(\mathbf{r}, t) = \sum_{j=1}^{N} \delta(\mathbf{r} - \mathbf{r}_j(t)),
\]
and calculate its Fourier transform

$$\rho_q(q, t) = \int e^{-iqr} \rho(r) dr = \sum_{j=1}^{N} e^{-iqr_j(t)}, \quad (4)$$

we can express the self-ISF as a Fourier transform of the density-density correlation function:

$$F_S = \frac{1}{N} \langle \rho_q(t) \rho_{-q}(0) \rangle. \quad (5)$$

It can be useful to rewrite self-ISF for identical particles:

$$F'_S = \langle e^{-i\Delta r} \rangle. \quad (6)$$

Suppose we have a solution with particles undergoing Brownian motion. Let us assume that we have a particle at time \( t = 0 \) in the neighbourhood of the origin. As time progresses it will perform a random walk to the proximity of point \( R \) with probability \( G_S(R, t) d^3r \). This kind of probability can be in most cases described by diffusion equation. Because of this, \( G_S \) is a very good approximation of a solution to the diffusion equation \[2\]

$$\frac{\partial}{\partial t} G_S(R, t) = D \nabla^2 G_S(R, t), \quad (7)$$

where \( D \) is the diffusion coefficient. In this case, \( G_S \) has a Gaussian shape. Fourier transform of this diffusion equation is:

$$\frac{\partial}{\partial t} F_S(q, t) = -iq^2 DF_S(q, t). \quad (8)$$

Solution for this equation, subject to boundary condition \( F_S(q, 0) = 1 \), is:

$$F_S(q, t) = e^{-q^2Dt}, \quad (9)$$

which is an important result for light scattering, valid in idealized case of Brownian motion of identical non-interacting spherical particles.

### 3 Dynamic Light Scattering

For a better understanding of differential dynamic microscopy (DDM) it is good to have some basic knowledge about dynamic light scattering (DLS) method. DLS (called also photon correlation spectroscopy) is an important technique in physics of soft matter for determining particle sizes between 3 and 3000 nm, flow velocities etc. In DLS, sample is illuminated with monochromatic laser light, which is scattered by the sample. A so called speckle pattern can be observed on the screen behind the sample. It is produced by the interference of scattered wavefronts. In order to see the speckle pattern far from the sample, a coherent source must be used. Suppose that the sample is a solution of particles undergoing Brownian motion. Then the speckle pattern will not be stationary and will constantly change because of thermal fluctuations of the refractive index. The rate of these changes depends on sample structure and dynamics.

The incident laser beam, polarized in direction \( \hat{n}_i \) can be thought of as a plane wave with initial wave vector \( k_i \), of size \( |k_i| = 2\pi n/\lambda_0 \), where \( n \) is the refractive index of the medium. The beam gets deflected in the medium for the angle \( \theta \), and the scattered wave vector is \( k_f \). We can say that the scattering is elastic and the incident and final vector are of same size. We define the scattering vector

$$q = k_i - k_f$$

as the difference between the initial and final wave vectors. The size of the scattering vector is

$$q = \frac{4\pi n}{\lambda_0} \sin(\frac{\theta}{2})$$

The light is sent through an analyzer with direction \( \hat{n}_f \) and collected by a photomultiplier in the far-field, consequently we are directly measuring spatial Fourier transform of the scattered intensity: \( I_S(q, t) \). By
changing the observing angle $\theta$, we are choosing the wave vector $q$ at which we are observing scattered intensity. Detector is connected to an auto-correlator, which calculates the auto-correlation function of the intensity. We can write the intensity auto-correlation function as:

$$G^{(2)}(q, \tau) = \langle I_S(q, 0)I_S(q, \tau) \rangle = \lim_{T \to 0} \frac{1}{T} \int_0^T I_S(t)I_S(t + \tau)dt,$$

where $\tau$ is the time delay. For $\tau = 0$, the signal is completely correlated, but correlation decays with increasing delay. Normally, the normalized intensity auto-correlation function is measured:

$$g^{(2)}(q, \tau) = \frac{\langle I_S(q, 0)I_S(q, \tau) \rangle}{\langle I_S^2(q) \rangle} = \frac{G^{(2)}(q, \tau)}{G^{(2)}(q, 0)}.$$

In a similar way we define normalized field auto-correlation function. Instead of intensity, scattered electrical field takes place in the equations:

$$g^{(1)}(q, \tau) = \frac{\langle E_S(q, 0)E_S^*(q, \tau) \rangle}{\langle I_S(q) \rangle} = \frac{G^{(1)}(q, \tau)}{G^{(1)}(q, 0)}.$$

Note that $G^{(1)}(q, \tau) = \langle E_S(q, 0)E_S^*(q, \tau) \rangle$. Intensity and field correlation functions are connected through Siegert relation [2]:

$$g^{(2)}(q, \tau) = 1 + |g^{(1)}(q, \tau)|^2,$$

which is valid if the scattered field is a Gaussian distributed variable. Because of this connection, we see that both field and intensity correlation functions hold the same information. We extract this information by writing down equation for the scattered field $E_S$ of $N$ particles at positions $\{r_j\}$:

$$E_S(q, t) = -E_0 e^{i(kR - \omega t)} \sum_{j=1}^N b_j(q, t)e^{-iqr_j(t)},$$

where $b_j$ is the scattering amplitude of the particle. We are interested in the auto-correlation function of the scattered field. For identical and independent particles it can be seen that auto-correlation function is proportional to the self-ISF from equation 6:

$$\langle E_S(q, 0)E_S^*(q, \tau) \rangle \propto \langle e^{-iq\Delta r} \rangle.$$

It turns out that in case of Brownian motion, normalized auto-correlation function $g^{(1)}(q, \tau)$ is the same as self-ISF:

$$g^{(1)}(q, \tau) = e^{-q^2D\Delta t} = e^{-\Delta t/\tau_c}.$$
This means that electrical field fluctuations are connected to the density fluctuations, more exactly to the fluctuations of dielectric constant of the sample. By measuring the intensity auto-correlation function at different angles and fitting the $g^{(2)}(q, \tau)$ data, one can extract diffusion constant or hydrodynamic radius from the slope of the $\tau(q)$ plot. This is the most basic use of DLS, the more advanced being studies of polydisperse or anisotropic samples.

4 Differential Dynamic Microscopy

DDM effectively merges microscopy and dynamic light scattering. Technique does not require to optically resolve the sample, so particles below sub-diffraction limit dimensions can be probed. Different to DLS, only an ordinary white-light microscope and a high speed camera, capable of around 100 fps or more is needed. Instead of a photomultiplier at a fixed angle, an array of pixels can measure many small angle signals at once, which is an advantage. But the speed of the camera limits how fast dynamics can be observed. In DDM, a video $I(x, t)$ is captured and Fourier analyzed to extract important information. Analysis is similar to DLS, but with a few differences, which is described in the following sections.

4.1 Basics

Let us first consider a two dimensional scattering object, with negligible thickness along the optical axis. According to Abbe’s theory of microscope image formation, the observed image is the interference pattern of the diffraction phenomena in the sample. The refractive index distribution in the sample can be decomposed in Fourier components, each labeled with spacial frequency $u_{obj}$, so the object to be imaged can be thought of as the superposition of those components. Each frequency component acts as a periodic diffraction grating and in paraxial approximation scatters light at angle $\theta = \sin^{-1}(u_{obj}/\lambda)$ with respect to optical axis. The diffraction pattern generates on an image plane a set of sinusoidal fringes with spatial frequency $u_i = \sin(\theta)/\lambda = u_{obj}$ that does not depend on $\lambda$, meaning that the speckle patterns generated by different $\lambda$ will fully superimpose at small angles. Therefore, Fourier component of the image directly corresponds to Fourier component of the object \[1\ 4\].

Figure 2: Two dimensional cross-section of microscope image formation. Refractive index distribution in the sample can be Fourier decomposed in different modes, each acting as a diffraction grating. Fourier mode of the sample directly corresponds to Fourier mode of the image, regardless of the wavelength of the light.
This allows us to study Fourier transform of the image intensity \( I(x, t) \), defined as

\[
\hat{I}(q, t) = \frac{1}{2\pi} \int \int I(x, t)e^{-iqx}dx,
\]

in order to learn about relevant statistical quantities. Scattering wave vector \( q = (q_x, q_y) \) is in this case a two-dimensional projection of the real scattering vector \( Q \) to the object plane. We assume that \( Q \approx q \) which is valid for small angle scattering (we already did some paraxial approximations earlier). In practice, the numerical aperture of the microscope objective limits the maximum angle of the scattered light that can still be detected. This also means that there is a cut-off in observable \( q \) vectors. In most of the light scattering experiments, temporal and angular behaviour of \( \hat{I}(q, t) \) is observed directly in the so called far-field region, but a good coherent source is needed in order to do so. This can not be achieved when using an ordinary microscope lamp. Because of low temporal coherence of the source, interference effects giving speckle pattern will not persist at long range. Therefore, the detector must be positioned close to the sample, in the so called near-field or deep Fresnel region, where transverse correlation properties of the scattered light are independent on the distance from the source \([5]\). In our case, the microscope objective transfers the near-field image to the camera, which cannot be placed directly onto the sample. In the near-field region it is possible to recover scattering pattern \( \hat{I}(q, t) \) just by applying Fourier transform to the camera image (equation (17)). Using a low coherence source has also some advantages, when samples are confined in thin cells. Small values of longitudinal coherence length minimizes Fabry-Perot interference fringes observed with a laser.

![Figure 3](image_url)

**Figure 3:** Scattered light in a DDM experiment is collected by an objective lens. In the near-field region one can reconstruct scattering pattern equivalent to traditional far-field in b) just by applying Fourier transform. In c) we can see that \( q \) is a projection of \( Q \) to an image plane. For small angles we assume \( Q \approx q \). Reproduced from [6].

Image analysis can be done in multiple ways, two of them are described in the following paragraph. First method is a direct calculation of the so called image structure function \( D(q, \Delta t) \), which is defined by the expectation value of the Fourier power spectrum of the difference images \( d(x, t_0, \Delta t) = I(x, t_0, \Delta t) - I(x, t_0) \). The other method is using the image correlation function \( G(q, \Delta t) = (\hat{I}^*(q, t_0)\hat{I}(q, t_0 + \Delta t))_{t_0} \), which is connected to \( D(q, \Delta t) \), but is significantly less demanding in terms of calculation time. Expectation values are taken over many statistically independent realizations of the signal and \( \Delta t \) is a time delay. Relation between the two can be derived in the following way:

The difference images can be Fourier transformed:

\[
d(q, t_0, \Delta t) = \hat{I}(q, t_0, \Delta t) - \hat{I}(q, t_0) = \Delta \hat{I}(q, \Delta t).
\]

Then, as said earlier, image structure function equals to average Fourier power spectrum of the difference images:

\[
D(q, \Delta t) = \langle |\Delta \hat{I}(q, \Delta t)|^2 \rangle_{t_0}.
\]

We can further write:

\[
\langle |\Delta \hat{I}(q, \Delta t)|^2 \rangle_{t_0} = \langle |\hat{I}(q, t_0 + \Delta t) - \hat{I}(q, t_0)|^2 \rangle_{t_0} = \langle |\hat{I}(q, t_0 + \Delta t)|^2 \rangle_{t_0} + \langle |\hat{I}(q, t_0)|^2 \rangle_{t_0} - 2\text{Re}(\langle \hat{I}^*(q, t_0)\hat{I}(q, t_0 + \Delta t) \rangle_{t_0}).
\]


Figure 4: Difference images $d(x, \Delta t)$ and the reconstructed image structure function $D(q, \Delta t)$ for three different time delays: 0.01, 0.1 and 1 s. The contrast of the speckles in difference images gets larger with increasing time delay. Images for $D$ seen here are result of averaging over 2000 independent difference images. Adapted from [5].

In the last term of equation $20$ we recognize the image correlation function $G(q, \Delta t)$. In an ergodic system, fluctuations at different times are statistically equal, so the first two terms in the right hand side of equation $20$ give a same value. We can conclude that the relation between the structure and the correlation function, valid for statistically stationary processes, is written as:

$$D(q, \Delta t) = 2[G(q, 0) - G(q, \Delta t)]. \quad (21)$$

Intensity correlations should decay with increasing time delay, so $G(q, \Delta t)$ is a decreasing function of $\Delta t$ from $G(q, 0)$ to 0, therefore according to equation $21$ $D(q, \Delta t)$ is an increasing function of $\Delta t$ from zero to $2G(q, 0)$. Essentially, structure functions are correlation functions turned upside down. Even if from theoretical point of view these two functions are equivalent, in practice they are not. Direct calculation of the structure function is much more time consuming, but is more robust and very effective at cancelling out time independent noise contributions. On the other hand, calculation of the correlation function $G(q, \Delta t)$ can be very fast and useful especially when real time processing is needed. In fact, $G(q, \Delta t) = \langle I^*(q, t)I(q, t + \Delta t)\rangle_{t_0}$ is an auto-correlation function and can be evaluated using Wiener-Khinchin theorem [2], which connects auto-correlation function with the Fourier power spectrum in the following way:

$$\int_{-\infty}^{\infty} I^*(t)I(t + \tau)dt = \int_{-\infty}^{\infty} |I_\omega|^2 e^{i\omega\tau} d\omega. \quad (22)$$

This implies that if we perform temporal Fourier transform on our signal, and calculate its absolute square, its inverse Fourier transform will be the needed auto-correlation function. In order for this to work properly, input signal must be zero padded, similarly to when convolving two signals. Full result of a linear convolution is always longer than any of the two input vectors. If we do not provide enough space to put the end of the convolution result, the convolution will be circular and results will be mixed. Proper amplitude normalization must be used at the end. Using this knowledge, $G(q, \Delta t)$ can be extracted very fast.

Final question persists: how are $G(q, \Delta t)$ and $D(q, \Delta t)$ connected to the dynamics of the system? We define normalized image correlation function as:

$$g(q, \Delta t) = \frac{G(q, \Delta t)}{G(q, 0)}. \quad (23)$$
We can also rewrite equation (21) to:

\[ D(q, \Delta t) = 2G(q, 0)[1 - g(q, \Delta t)]. \] (24)

If we assume that intensity fluctuations are proportional to fluctuations of sample density, we recognize in \( g(q, \Delta t) \) the self-intermediate scattering function \( F_S = \exp(-q^2 D \Delta t) = \exp(-\Delta t/\tau) \), where \( \tau = 1/Dq^2 \).

This means that for Brownian motion, every Fourier density mode decays with characteristic time \( \tau(q) \). The final form for image structure function, used for fitting the experimental data is then \[ D(q, \Delta t) = A(q)[1 - e^{-\Delta t/\tau}] + B(q). \] (25)

The term \( B(q) \) was added to account the noise of the detection system and \( A(q) \) is related to scattering properties of the sample, properties of the microscope objective and light source. Their prior knowledge is not obligatory for performing DDM experiments and can be treated as fitting parameters beside \( \tau \) once \( q \) is fixed.

4.2 Experiment

A brief overview of an experiment done in \[1, 5\] is presented in this section. Experimental setup was composed from an ordinary white-light microscope, equipped with a complementary metal-oxide-semiconductor (CMOS) camera with pixel size of 12 \( \mu m \). Sample was a capillary tube, filled with aqueous dispersion of polystyrene spheres with 73 nm in diameter. Low enough mass concentration of 1\% ensured that the particles can be thought of as non-interacting. Sample was illuminated with white light, focused by a condenser with numerical aperture \( NA = 0.9 \), and observed through an objective with magnification \( 63 \times \) and \( NA = 0.7 \). Accounting the magnification, effective pixel size was 0.19 \( \mu m \). A video sequence of 1000 images was taken with frame rate 100 fps and exposure time 1 ms. Image structure function \( D(q, t) \) was calculated directly through image subtraction into difference images and applying fast Fourier transform. When sample is isotropic, \( D(q, t) \) is rotationally symmetric (see figure 4). That means that azimuthal averaging can be performed for a chosen \( q = \sqrt{q_x^2 + q_y^2} \). For each \( q \), data was fitted using equation 25 and extracted characteristic times \( \tau = 1/Dq^2 \) were plotted as a function of \( q \). In the Fig. 5(a), fitted image structure function is seen for three different values of \( q \) (2.6 \( \mu m \)\(^{-1}\), 3.9 \( \mu m \)\(^{-1}\), 4.5 \( \mu m \)\(^{-1}\)). Fit for each \( q \) yielded a value for characteristic time \( \tau \). Points \( \tau(q) \) are plotted in Fig. 5(b), together with DLS data of the same particles. Result for diffusion constant \( D \), calculated by fitting the \( \tau(q) \) data is \( D = 6.2 \pm 0.3 \mu m/s^2 \). Using Einstein-Stokes relation \( D = k_B T/(3\pi\eta d) \), where \( d \) is the diameter, one can also calculate a prediction for diffusion constant \( D = 5.98 \mu m/s^2 \), so the experimental result is in good agreement with theory.

![Figure 5: a) Growth of averaged image structure function (ISF) with increasing time delay, plotted at three different values of \( q \): (2.6 \( \mu m \)\(^{-1}\), 3.9 \( \mu m \)\(^{-1}\), 4.5 \( \mu m \)\(^{-1}\)). ISF increases quickly at short delays and reaches a plateau at longer delays. b) Data for extracted \( \tau(q) \) for aqueous dispersion of 73 nm particles. Black dots are DDM data and red dots are DLS data. The red line represents theoretical prediction from Einstein-Stokes formula. We see that the two methods are somehow complementary, DDM working at smaller angles and DLS at larger. Reproduced from [1] and [5].](image-url)
4.3 Generalization to three dimensions

The mentioned scattering wave vector \( Q \) (section 4.1) is in fact three-dimensional: \( Q = (q, q_z) \). When using poly-chromatic or incoherent light source, there is a distribution of incident and scattered wave vectors \( k_i \) and \( k_s \). This means that scattered intensity at vector \( q \) is a weighted sum over all possible combinations of incident and scattered wave vectors that correspond to the vector \( q \). In other words, scattering processes with different \( q_z \) can contribute to the same vector \( q \), as seen in figure 6. In order to properly evaluate scattered light, a more complex method of calculation must be used. Detailed explanation is done in [5], where Nemoto-Streibl model of a microscope is used:

\[
I(x, t) = I_0 + \iiint dx'dz'K(x - x', -z')\rho(x', z', t),
\]

which means that final intensity is a linear superposition of contributions from all the sample layers. Each contribution is a convolution between density and transfer function \( K \), where \( K \) is modelled in a way that all contributions from microscope and light source properties are taken into account. The intensity can then be connected to sample properties via the auto-correlation function \( G(q, \Delta t) \) and the self-ISF from equation [5]

\[
G(q, \Delta t) = \int dq_z |K(q, q_z)|^2 F_S(q, q_z, \Delta t).
\]

The 3D generalization of the normalized auto-correlation function is then \( g(q, \Delta t) \approx g_{2D}(q, \Delta t)g_z(q, \Delta t) \), where \( g_{2D}(q, \Delta t) = e^{-q^2 D \Delta t} \) and \( g_z(q, \Delta t) \) is the correction. If the numerical aperture of the microscope condenser lens is greater than the numerical aperture of the objective and wavelength spread \( \Delta \lambda \ll \lambda_0 \), where \( \lambda_0 \) is the central wavelength, then: \( g_z \approx 1 \) and \( g(q, \Delta t) \approx g_{2D}(q, \Delta t) \), so equation [25] is still a very good approximation.

\[Q_2\]
\[Q_1\]
\[k_{s1}\]
\[k_{s2}\]
\[k_1\]
\[k_2\]
\[q_1\]
\[q_2\]
\[q_{z1}\]
\[q_{z2}\]

**Figure 6:** Effect of a spatially incoherent light source. A pair of different \( k_i \) vectors with the same size but different directions can give rise to the same vector \( q \). The two vectors have different value of \( q_z \). Similar thing can happen when two vectors \( k_i \) are parallel, but have different sizes. This is when a polychromatic light is used. Reproduced from [5].
5 Conclusion

DDM is an effective and robust technique for performing DLS experiments with a camera equipped bright-field microscope. It operates at small scattering angles in the near-field region. A series of images is taken with a high-speed camera and Fourier analysis is performed to extract the intermediate scattering function, similarly to DLS. Its key advantage is that it requires no special experimental requirements and can be performed in any laboratory that has a microscope. It is a good complementary technique to DLS because it works at smaller angles, but is limited in speed by the camera frame rate. It is appropriate for observation of slower dynamics, mostly thermal fluctuations of soft matter and also dynamic heterogeneities. This includes liquids and gels made of colloids, polymers and liquid crystals and also biological samples, like bacteria and cells. For example, technique was already used for observations of colloidal aggregation \[7\], swimming speed and bacterial motility in \textit{Escherichia coli} suspensions \[8\] and dynamics of anisotropic colloids \[9\]. Using polarized DDM (pDDM), one can also extract viscosities and elastic constants of liquid crystals, only based on thermal fluctuations \[6\]. Technique is also applicable to fluorescence microscopy. DDM is a step forward in the studies of soft matter and many scientific fields could make use of it in the future.

References


